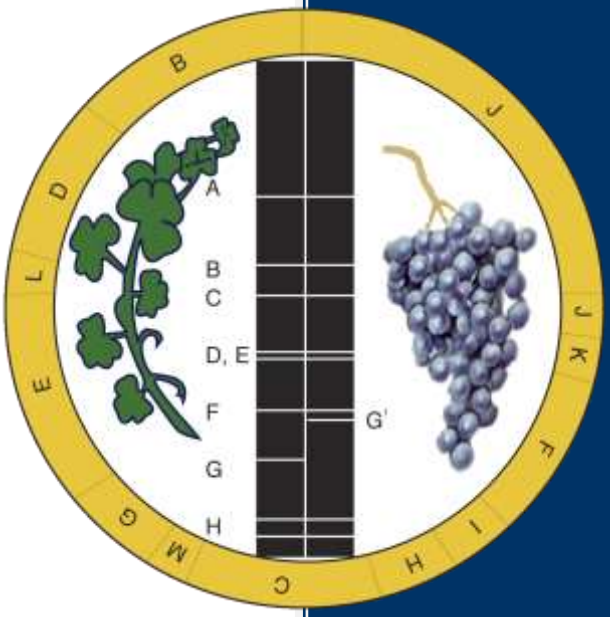
# ANNUAL REPORT **2018**




# National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

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# INTRODUCTION

This annual report details the work of the National MRSA Reference Laboratory in 2018. Throughout 2018, the laboratory continued to deliver on its role in assisting medical professionals is the control of MRSA in hospitals and the community in Ireland.

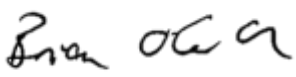
In addition to the routine work of the laboratory which includes molecular typing for epidemiological investigation and the detection of important virulence factors, service developments and projects during the year included:

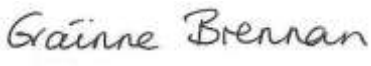
- the expansion of molecular tests to investigate linezolid resistance in coagulase negative staphylococci (CoNS) and enterococci;
- further characterisation of community associated (CA) and livestock associated (LA)- MRSA and Meticillin susceptible *S. aureus* (MSSA) recovered in Ireland using DNA microarray;
- Characterisation of *S. aureus* isolates recovered from healthcare workers, patients and their environment;
- the staff of the laboratory continued to provide education and training to doctors, nurses and scientists and contribute to MRSA research by completing/collaborating in numerous publications.

The laboratory also began evaluating ways in which whole genome sequencing may provide users of the laboratory with assistance when investigating outbreaks caused by MRSA. To date several outbreak incidents have been investigated using this technology and the laboratory hopes to further expand this service in the near future.

We would like to thank the staff of NMRSARL who continue to work tirelessly to provide the service; our collaborators in research and development which yields a fruitful new knowledge on MRSA and the Laboratory Medicine Directorate and St. James's Hospital for continuing to support the NMRSARL in the important work that it provides.

We hope that you find the following pages useful and informative.





Dr. Brian O'Connell
Director

Dr. Gráinne Brennan
Chief Medical Scientist

## SUMMARY

**Public health impact**

- The surveillance and identification of potential outbreaks of MRSA and MSSA
- The laboratory also monitors the incidence of *pvl* carrying strains of *S. aureus* and the strains associated with healthcare infections

**New service developments**

- Phenotypic and genotypic methods remain under constant review to take advantage of any newly developed methodologies;
- Investigation of transferrable resistance genes encoding linezolid resistance in enterococci and CoNS

**Activity**

- During 2018, the EARS-Net project accounted for 15.9% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 30.5%
- Further increase in the uptake of newer services including DNA microarray profiling of *S. aureus* and investigation of linezolid resistance among Enterococci and CoNS

**Research and development**

- The laboratory continues to participate in numerous projects and is collaborating with the Dublin Dental University Hospital, Trinity College to evaluate the potential of whole genome sequencing for routine use

**Education and training**

- The laboratory continues to offer training to biomedical science students and postgraduate students in Trinity College Dublin and Dublin Institute of Technology

**Future developments**

- As technology expands into whole genome sequencing, this technology will replace a number of the current assays and produce definitive data on the similarities and differences between organisms

## ROLE OF THE LABORATORY

Since its establishment in 2002, the Laboratory has supported efforts to prevent and control MRSA in Ireland by providing expertise to laboratories in the correct identification of *Staphylococcus aureus* isolates, by tracking circulating strains as part of infection control, by detecting the emergence of new mechanisms of resistance to antibiotics, by screening for the presence of novel virulence factors or toxins, and by participation in research and development initiatives at home and abroad.

## SERVICES

The NMRSARL provides the following services:

- Investigation of MRSA isolates using phenotypic and molecular techniques for the following reasons:
  - confirmation of *S. aureus* identity
  - epidemiological typing (including *spa* typing)
  - detection of resistance and virulence genes including *pvl, mec, nuc, eta, etb* and *etd*
- Investigation of meticillin susceptible *S. aureus* (MSSA) isolates
  - For the detection of the *pvl* and exfoliative toxin genes
  - Outbreak investigation of strains using *spa* typing
- Advice
  - on treatment and management of patients with MRSA through its medical director
  - on infection control through the infection control team of SJH
  - on laboratory aspects of MRSA through the scientific staff of the laboratory

## ISOLATES

Isolates, recovered from patients attending community medical practitioners or hospitals, are submitted to the laboratory from all hospital microbiology laboratories throughout the Republic of Ireland.

In addition to this the NMRSARL also provides laboratory support for the MRSA component of EARS-Net in Ireland. All Irish hospital laboratories participating in EARS-Net send MRSA isolates from blood cultures (one per patient per quarter) to NMRSARL where they are investigated for resistance to oxacillin, vancomycin and teicoplanin using standard E-test or E-test™ macro-method techniques. NMRSARL also provides data on rates of resistance to other clinically useful antibiotics.

# PUBLIC HEALTH IMPACT

The impact of the various activities of the NMRSARL on public health is described below.

| Organism | Activity | Number of isolates | Outcome |
|---|---|---|---|
| **MRSA blood culture isolates** | Surveillance | 115 | Participation in EARS-Net which is a European wide network of national surveillance systems, providing European data on antimicrobial resistance for public health purposes |
| **MRSA & MSSA** | PVL toxin testing | 531 | Surveillance, recognition, investigation and management of PVL *S. aureus* in Ireland |
| **MRSA & MSSA** | Surveillance analysis and identification of trends | 622 | Typing and susceptibility testing of MRSA and MSSA isolates submitted throughout the year. |
| **ST1-t127-MRSA-IV** | Surveillance | 41 | Investigation of isolates recovered from community and healthcare sources between 2013 and 2016 in order to investigate the isolate relationships and the extent of their spread |
| **Mupirocin resistant t127-MRSA-IV** | Surveillance | 8 | Ongoing surveillance of mulitantibiotic resistant strain which was initially limited to one hospital but which has since spread to other hospitals and the community |
| **MRSA and MSSA** | Surveillance | 214 | Outbreak/cluster investigations (n=69) throughout Ireland |
| **MRSA and MSSA** | Confirmation of resistance against various antibiotic agents | 469 | Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents. |
| **VRE and CoNS** | Confirmation of linezolid resistance | 48 | Characterisation of resistance mechanism associated with increased linezolid resistance in VRE and CoNS |
| **MSSA & MRSA** | Characterisation of *S. aureus* recovered from healthcare workers | 393 | Determine prevalence of *S. aureus* among healthcare workers in Irish hospitals. |
| **LA-MRSA** | Surveillance | 8 | Characterisation of MRSA strains recovered from humans but with known association to livestock including isolates harbouring the *mecC* gene. |

## REFERENCE LABORATORY WORK

During 2018, work under the EARS-Net project accounted for 13% of the overall workload of the NMRSARL while MSSA isolates and non *S. aureus* isolates accounted for 30.3% (Fig. 1) In recent years an increase in requests for investigations of MSSA isolates has led to a change in the services of the laboratory and 2018 saw a further increase in the uptake of newer services including DNA microarray profiling of *S. aureus* and investigation of linezolid resistance among Enterococci and CoNS.

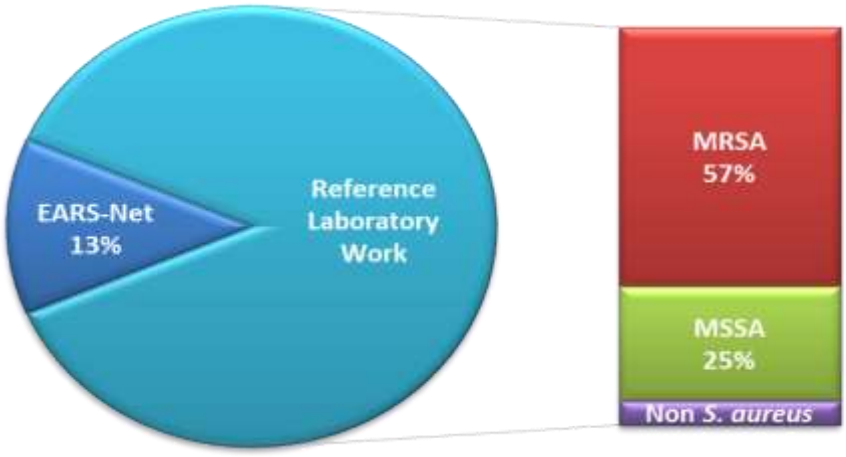



Fig 1 Workload of the NMRSARL during 2018

Along with a steady increase in the number of isolates submitted, the complexity of tests has also increased over time. Currently the laboratory performs phenotypic investigation on all isolates submitted however further molecular investigation is performed on over half of the isolates including investigation for PVL toxin (n=545) or *spa* typing (n=503). This change is primarily due to the changing epidemiology of MRSA circulating in Ireland and the limited information that can be obtained from phenotypic investigation of these emerging strains.

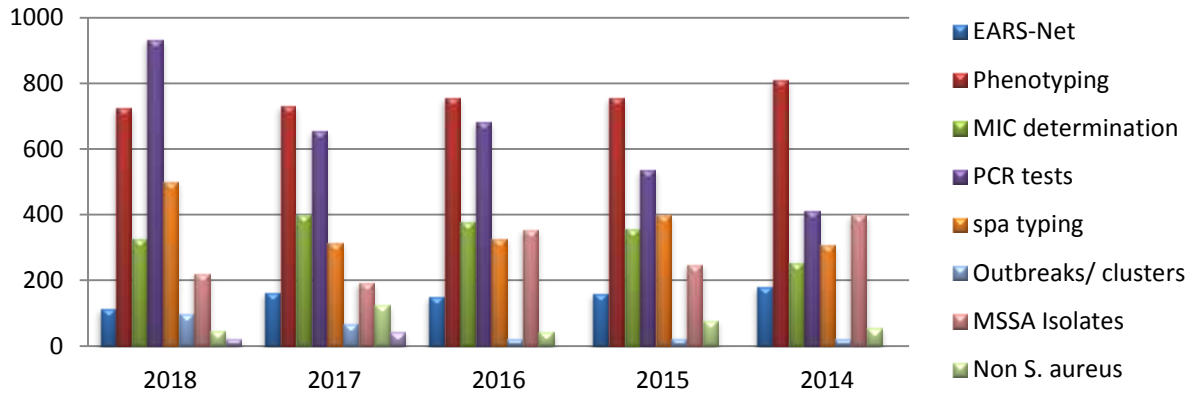



Fig 2 Distribution of workload throughout 2018

## Linezolid resistance in Staphylococci and Enterococci

In 2018 Ireland had the second highest proportion of vancomycin resistant *Enterococci faecium* in Europe. In addition, in recent years an increase in resistance to linezolid has also been reported (1). Since 2016 the NMRSARL has investigated linezolid resistance in Enterococci and Staphylococci for the presence of *cfr* and *optrA* (2). Furthermore, following a report of an additional resistance gene *poxtA*, the NMRSARL retrospectively investigated all isolates submitted in 2018 for this gene (3).

Linezolid is often the drug of last resort to treat serious infections caused by Gram-positive cocci. While resistance frequently arises due to mutations in the 23S rRNA gene, altering the drug binding site, and/or the 50S ribosomal proteins L3, L4 and L22, impairing linezolid binding, less frequently it has also been associated with the acquisition of a plasmid-encoded methyltransferase gene *cfr* or ABC transporter gene *optrA.* The presence of *cfr* can result in the PhLOPS$_A$ phenotype i.e., resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A compounds, due to their overlapping binding sites. In contrast however, *optrA* confers resistance to oxazolidinones and phenicols only while, along with these *poxtA* also encodes resistance to tetracyclines.

Work carried out in the NMRSARL has found isolates recovered in Ireland can carry multiple resistance mechanisms. In 2017 an *E. faecium* isolate was found to harbour both *cfr* and *optrA* while in 2018 an isolate was found to be positive for *optrA* and *poxtA.*

While the numbers investigated to date remain low, since introduction approximately 8% of isolates investigated have found to harbor the *optrA* gene. However while in 2016 a near equal proportion of *E. faecalis* and *E. faecium* were found to harbor the gene, in 2017 *optrA* was detected in only a single *E. faecium* with the remaining positive isolates identified as *E. faecalis* (Fig. 3).

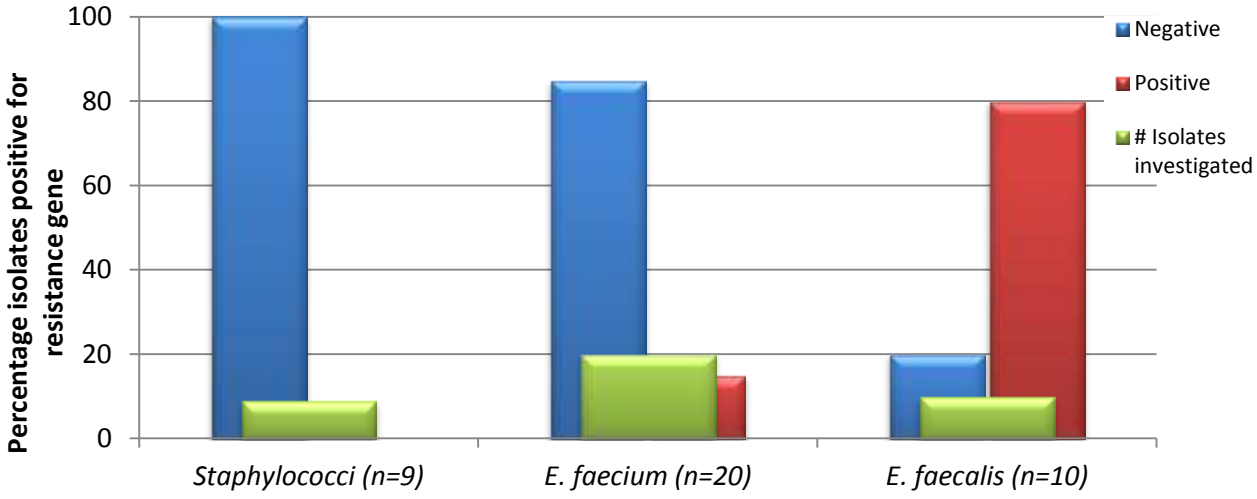



**Fig 3 Distribution of linezolid resistant isolates investigated in 2017. While E. faecalis accounted for the majority of isolates tested, only one was optrA positive while 42.9% of E. faecium isolates investigated were found to be positive**

## PVL positive *S. aureus*

Throughout 2018 the detection of PVL continued to be the most frequently requested test. The PVL toxin is a cyto-toxigenic toxin produced by *S. aureus* which is clinically associated with skin and soft tissue infections but is rarely reported in isolates recovered from invasive infections. In 2018, 531 *S. aureus* isolates (non-BSI) were investigated for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL and representing an increase of 23.3% from 2017. The isolates investigated included 327 MRSA and 202 MSSA.

Among the MRSA isolates 31.2% (102/327) were found to be positive while 11.8% (24/202) of MSSA isolates were also positive.

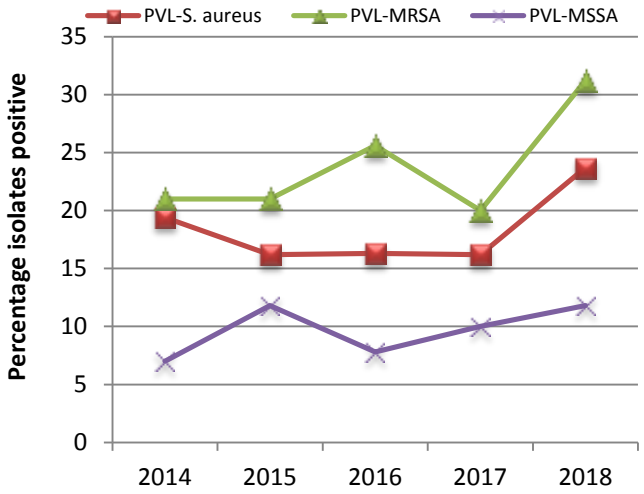



Fig 4 Percentage of isolates positive for PVL in 2018

The significant increase in the number of PVL-positive MRSA during 2018 is due primarily to an increase in the number of outbreaks and clusters identified during the year. The NMRSARL were advised of nine family clusters involving at least two family members along with five hospital outbreaks involving at least two patients in several hospitals. In addition the laboratory was advised of several incidences where patients died due to PVL-positive MRSA infections.

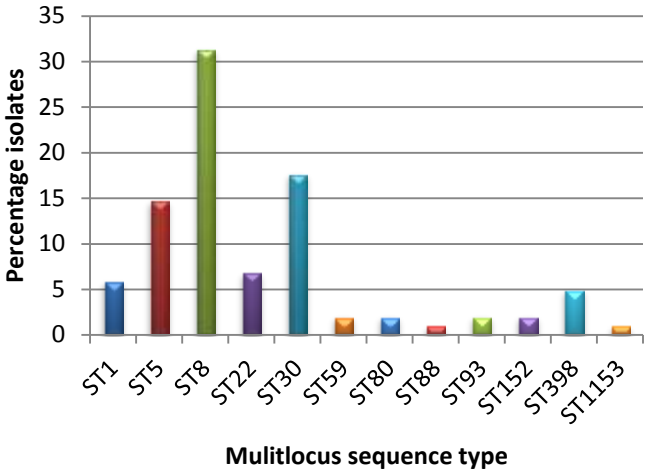



Fig 5 Distribution of sequence types among PVL positive MRSA

The PVL-positive MRSA population continues to be less diverse with 63% of the isolates associated with only three types (ST8, 31.4%; ST30, 17.6%; ST5, 14.7%). While many of the PVL-positive MRSA strains have been recognised in Ireland for a number of years, ST398 has not previously been found. This strain is mainly considered a livestock associated strain, where previous reports have described asymptomatic carriage in persons exposed to occupational hazards (e.g., veterinary personal and pig farmers). Reports of PVL-positive ST398 are relatively rare and where present are associated with severe skin and soft tissue infections.

Among the isolates recovered from blood stream infections only 5% were PVL positive (5/115) however all of these were assigned to different MLST.

## ANTIMICROBIAL RESISTANCE AMONG MRSA IN IRELAND

The phenotypic epidemiological typing techniques used in the NMRSARL enables the laboratory to monitor resistance among MRSA strains against clinically useful antimicrobial agents and to identify emerging resistance that may cause concern into the future with the EARS-Net isolates providing a representative collection of isolates recovered throughout the country. The current predominant strains circulating in Ireland (ST22-MRSA-IV) exhibits a non-multiantibiotic resistant susceptibility profile. However the emerging community associated strains carry multiple virulence and resistance genes including those associated with aminoglycoside and tetracycline resistance.

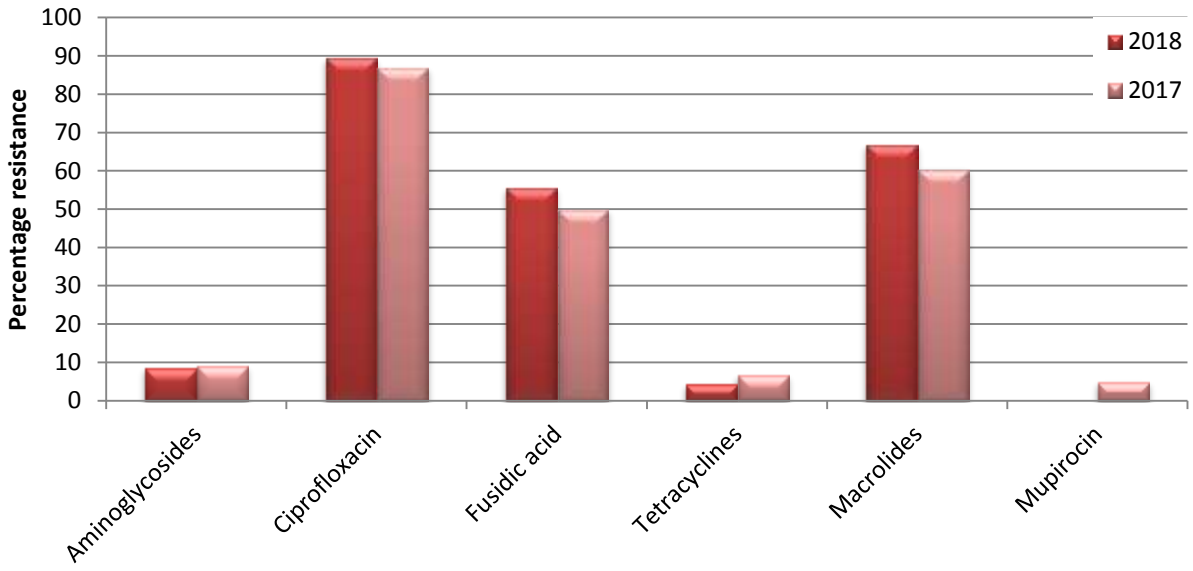



Fig 4 Resistance rates among EARS-Net isolates recovered in 2018

The increase in resistance to fusidic acid is a trend which has been observed over a number of years having increased from 27% in 2006 to the current level of 53% and is a worrying concern since fusidic acid (FA) remains a clinically useful antimicrobial for difficult to treat skin and soft tissue infections.

Among the FA-resistant isolates, 79.7% are ST22-MRSA-IV and resistance among these isolates is predominantly associated with mutations in the *fusA* gene. Resistance among the remaining isolates is associated with the *fusC* gene.

The *fusC* gene is located either (i) within a SCC element, either alone or adjacent to a SCC*mec* element where it forms part of a composite island (CI), or (ii) within a SCC*mec* element, where it is termed a chimeric element due to the presence of *mecA* and *fusC* within a single SCC*mec* element.

Work in the NMRSARL has found extensive genetic diversity among *fusC*-positive MRSA isolates in relation to their genetic backgrounds and the SCC*mec*-*fusC* elements that they harboured and the NMRSARL is currently collaborating with colleagues in the Dublin Dental University Hospital to further investigate these isolates.

## Antimicrobial susceptibility among MRSA recovered from non- blood stream infections

While the previously mentioned rates of resistance relate only to EARS-Net isolates, a greater proportion of the work in the NMRSARL relates to isolates recovered from non-blood stream infections. In addition these isolates are often recovered from patients in the community where no risk factors for MRSA infection are present.

These isolates are submitted from different users on an ad hoc basis and therefore do not represent true prevalence characteristics of strains in the community. However it is possible to determine resistance profile of the isolates that were selected for submission to the NMRSARL.

Below shows the profile of all non-BSI isolates investigated in comparison to those of BSI isolates. Typically in Ireland ST22-MRSA-IV is the predominant HA-MRSA accounting for 80% of MRSA investigated under the EARS-Net project and exhibits a non-multiantibiotic resistant profile. However the non-BSI isolates recovered both in healthcare facilities and in the community, and which may also be among others, ST22-MRSA-IV, exhibit higher levels of resistance against the panel of antibiotics tested with 74% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics and in particular to aminoglycosides, mupirocin and tetracycline.

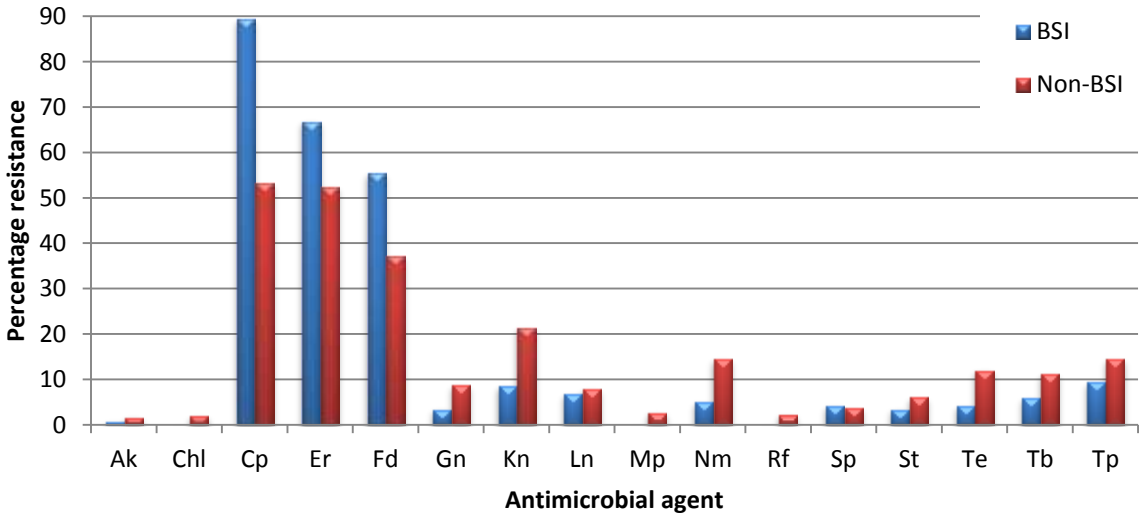



**Fig 8 The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent in comparison to those recovered from non-blood stream infections.**

**Resistance patterns determined for MRSA isolates by antibiogram- resistogram typing. Percentage for each agent includes those exhibiting resistance as determined in accordance with EUCAST or in-house developed interpretive criteria. Abbreviations: Ak; amikacin, Chl; chloramphenicol, Cp; ciprofloxacin, Er; erythromycin, Fd; fusidic acid, Gn; gentamicin, Kn; kanamycin, Mp; mupirocin, Nm; neomycin, Sp; spectinomycin, St; streptomycin, Te; tetracycline, Tb; tobramycin, Tp; trimethoprim.**

# ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

Like Europe, ST22-MRSA-IV is the pandemic clone in Ireland and, in 2018, was associated with 73.7% of MRSA causing blood stream infections. This strain is known also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13, or Canadian MRSA-8 (4)

This strain has been reported in many countries and, where present, tends to be the predominate strain accounting for >50% of MRSA in Portugal, and Malta and in England it is currently associated with 85% of bacteraemia cases. The strain occurs in hospitals as well as among outpatients in the community but it has also been recovered from companion animals such as horses, cats and dogs (4).

Common resistance patterns exhibited by the ST22-MRSA-IV strain include resistance to fusidic acid, ciprofloxacin, and erythromycin. Variable virulence markers in ST22-MRSA-IV are *sec* and *sel* as well as the IEC genes encoded by lysogenic β-haemolysin-converting phages (*sak, chp, scn*) (4).

In Ireland in 2018 the most frequently occurring *spa* type among the ST22 isolates, t032 continued to be the most predominant strain however other ST22 associated *spa* types included t022, t515, t223, t2945 and t020 were also recognised.

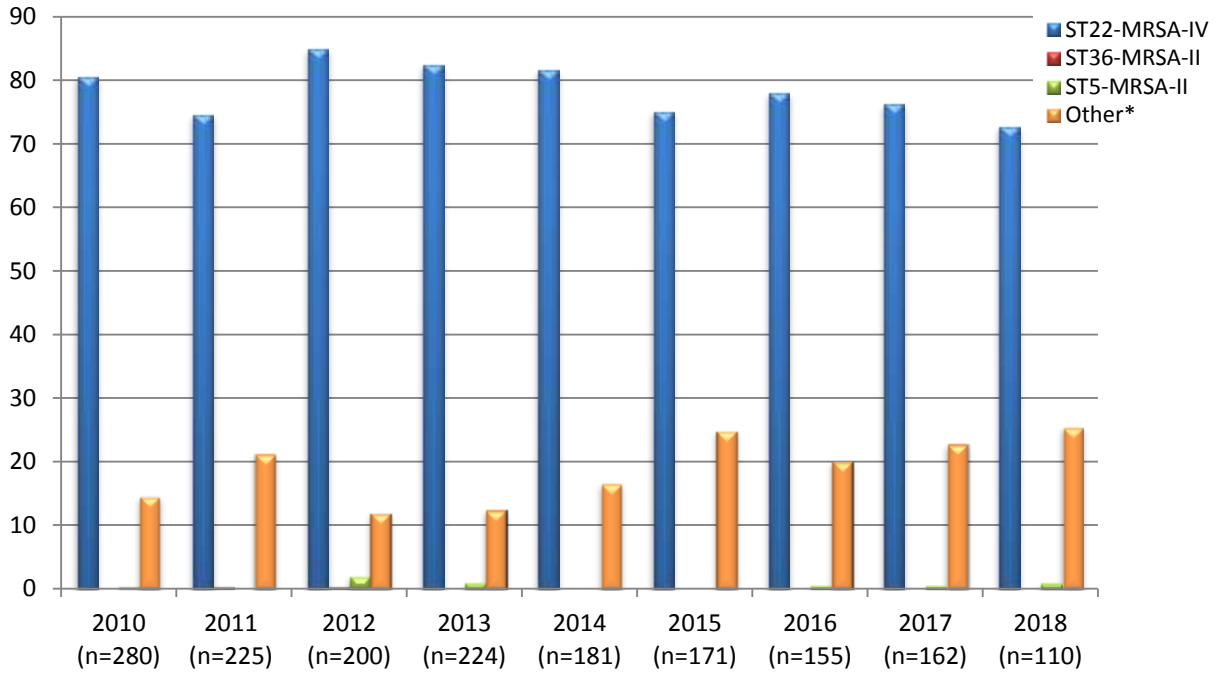



**Fig 9 Epidemiological types of MRSA strains recovered from blood stream infections inferred using antibiogram resistogram (AR) typing during 2018. During the year historical AR types AR13 and AR14 (both associated with ST8-MRSA-II) were not detected among EARS-Net isolates. The total number of isolates investigated each year is shown in parentheses.**

***Further molecular analysis of isolates categorized as 'other' assigned isolates to numerous sequence types however predominant STs included ST1, ST5 and ST8 all carrying SCC*mec*IV.**

# MOLECULAR EPIDEMIOLOGICAL TYPING OF MRSA

Typing methods for discriminating different bacterial isolates are essential epidemiological tools in infection prevention and control. Traditional methods based on phenotypic characteristics have been used for many years however often fail to provide sufficient discrimination of isolates in outbreak situations. In addition, the acquisition of other resistance mechanisms, along with the emergence of newer MRSA strains has led the NMRSARL to explore other typing methods to allow easier comparison of MRSA recovered in Ireland.

*spa* typing involves sequencing of the Staphylococcal protein A gene (*spa)* to recognise mutations or repeat insertion/deletion events that can cause changes in the polymorphic X region of the *spa* gene. It has become a well-established discriminatory method for outbreak investigations but has also been shown to be useful for long-term epidemiological studies. The availability of MLST data associated with *spa* types on an online database facilitates comparison of Irish isolates with isolates from all other countries. Based upon repeating patterns (BURP) analysis clusters *spa* types together based on the repeat succession pattern of *spa* types (5) .

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for over 54% of the isolates and, similar to previous years, included ST1, ST5, ST8 and ST30 (Fig 6). A change was also observed in the predominant strain which in recent years had been ST1 but in 2018 was ST5. This strain has been associated with an outbreak in 2018 but was also recovered from a number of patients in the community and in other healthcare facilities. The majority of the isolates were PVL negative (74%) however the remaining were PVL and associated with t002.

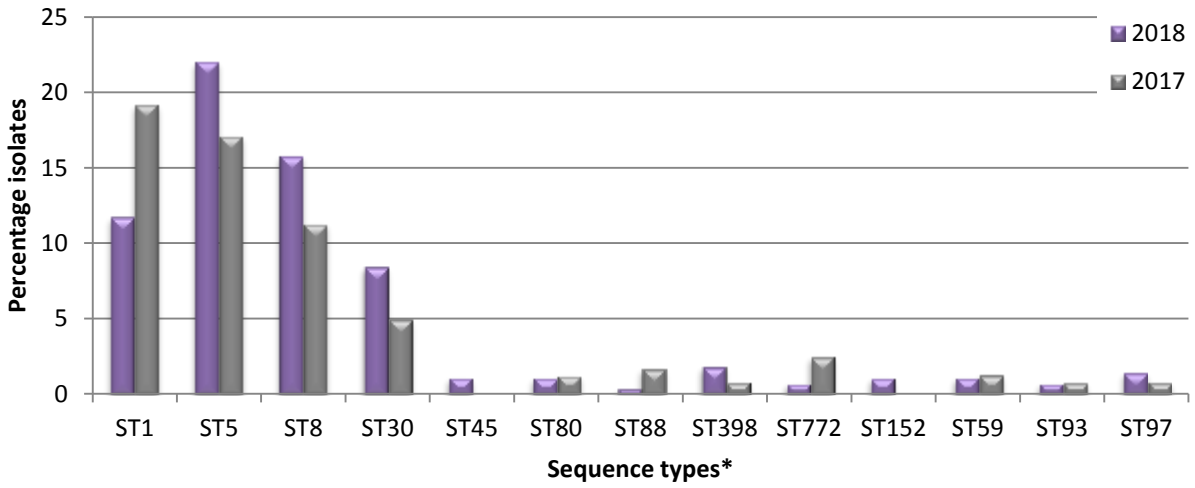



**Fig 10 Most frequently recognised MLST among MRSA isolates investigated by *spa* typing during 2018**

***Sequence type inferred from data available on the Ridom *spa* typing database**

# EMERGING STRAINS OF MRSA IN IRELAND

Previously MRSA was clearly defined as healthcare associated (HCA-) , community acquired (CA-) or livestock associated (LA-) however the lines of separation are becoming increasingly blurred with the importation of CA-MRSA strains into healthcare facilities and the zoonotic spread of LA-MRSA to humans. Furthermore, in Ireland over time, a strain displacement has occurred resulting in the ST22-MRSA-IV predominating in healthcare facilities. This displacement has also been reported in other countries where, once community associated strains have now become the predominant hospital associated strains (USA 300 in America and ST772 in India). Many of the strains recognised in Ireland have been reported elsewhere and very often, these strains exhibit greater resistance and harbour more virulence genes than the ST22 strains and so close monitoring is required in order to control the spread of these strains in the hospital setting.

## *mecC* mediated LA-MRSA

Since 2011, *mecC* MRSA has been reported in MRSA recovered from humans, livestock, wild animals and companion pets throughout Europe. While *mecC* had not been identified in Ireland since 2010, in 2017 there were four isolates harbouring *mecC* submitted to the laboratory. However in 2018 only one *mecC* isolate was submitted to the laboratory for investigation. Similarly to previously reported *mecC* isolates, this one was spa type t843 and lacked many of the human associated virulence genes usually found in MRSA.

## CC398-MRSA

First reported among pigs in the Netherlands in the early 2000s CC398 LA-MRSA has since been reported among a range of livestock and horses, as well as in humans in several European countries along with America and Australia. In addition some reports suggest that CC398 MRSA accounts for up to 25% of all community-associated MRSA in some parts of Europe. Although first detected in 2012 in Ireland, CC398-MRSA continues to be relatively rare with only two incidences detected in 2017. Furthermore, although traditionally associated with livestock, one of the isolates harboured the PVL genes suggesting human adaptation of the strain.

# WHOLE GENOME SEQUENCING TO INVESTIGATE OUTBREAKS CAUSED BY CA-MRSA LINEAGES

Molecular typing techniques have been highly useful in the monitoring the spread of MRSA strains in healthcare facilities. However often such strains cannot be sufficiently differentiated by means of traditional DNA based methods. More recently, improvements in both the time-to-results and the affordability of whole-genome sequencing (WGS) have allowed the NMRSARL to explore this technology in the typing of strains causing outbreaks in Irish healthcare facilities.

During 2017, the NMRSARL published results of a study of ST1-t127-MRSA-IV isolates recovered from community and healthcare sources between 2013 and 2016 in order to investigate the isolate relationships and the extent of their spread. In 2018, in conjunction with collaborators, we investigated another emerging CA-MRSA strain, CC88-MRSA-IV. This strain has been present in Ireland for a number of years however was recently associated with two outbreaks in a single hospital.

In this study whole genome sequencing revealed that, although isolate *spa* types and recovery dates suggested that two different CC88-MRSA strains may have been involved in the outbreaks in this healthcare facility, the outbreaks were caused by the same CC88/ST78-MRSA-IVa strain, which spread within the ward during two separate transmission periods. In addition it was also possible to highlight both the involvement of a HCW in the outbreak transmission chain and the strain's spread to two other Irish hospitals. A comparator study with a collection of international isolates also showed that the outbreak strain was most likely imported from Australia, where it is among the prevalent MRSA clones. This study also identified a second CC88-MRSA clone present in Irish hospitals, ST88-MRSA-IVa, which was likely imported from Africa, where it is predominant, and/or a country with a large population of African ethnic origin (6).

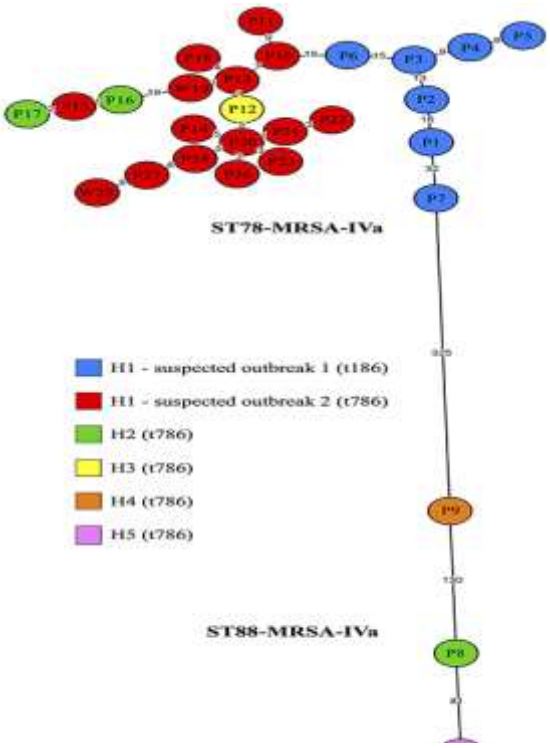



**Figure 11 A minimum spanning tree based on wgMLST profiles of CC88-MRSA isolates recovered between 2009 and 2017. Suspected outbreak 1 occurred in the NICU of H1 between 2009 and 2011. Suspected outbreak 2 occurred in the same NICU between 2014 and 2017. While one isolate was included per patient, two isolates (W19 and W28) recovered two years apart were included from a single healthcare worker. The remaining isolates were recovered from four different Irish hospitals** (6).

Along with CC88-MRSA-IV, WGS was also used to investigate three other outbreaks caused by CA-MRSA lineages, t002-CC5-MRSA-IV and t008-CC8-MRSA-IV, both of which are PVL positive strains. In the case of CC8-MRSA-IV, two outbreaks were identified at the same time in separate hospitals. While *spa* typing and DNA microarray were unable to distinguish the strains, WGS revealed that the clusters of isolates in each hospitals differed from each other by 54 single nucleotide polymorphisms (SNPs) confirming that they were unrelated to each other.

We would like to thank collaborators in the DDUH for performing the WGS throughout the year and assisting in the data interpretation.

## EDUCATION

The NMRSARL plays a prominent role in the education of laboratory staff and clinical staff.

In particular, NMRSARL staff gave lectures to undergraduate and post graduate students in the Dept. of Clinical Microbiology, TCD and the Dublin Institute of Technology. Scientific staff shared techniques used in the NMRSARL with staff from other hospital laboratories, research facilities, undergraduate students, transition year students and provided expert knowledge to students of other laboratories completing higher degrees.

The laboratory also facilitated two post graduate students undertaking projects as part of Masters of Science which involved investigating the prevalence of *S. aureus* among inmates in an Irish prison and also the characterisation of the isolates recovered from these inmates.

## CONTINUOUS PROFESSIONAL DEVELOPMENT

The level of expertise and knowledge among staff of NMRSARL is maintained through the participation of staff at both national and international meetings, workshops and conferences. Throughout the year all staff continued their professional development through attending some of the following meetings;

- Journal clubs
- Focus on Infection
- Antimicrobial Resistance
- Microbiology Advisory Body

NMRSARL staff also ensured mandatory training requirements were met in areas such as;

- Risk Management
- Chemical safety awareness
- Manual Handling & Fire safety
- Quality Management
- Hand Hygiene
- Transport of patient specimens

# RESEARCH HIGHLIGHTS

NMRSARL continues to participate in several collaborations with both local and international groups in order to enhance the research in the field of *S. aureus* in Ireland.

### Whole genome sequencing

- Evaluation of core genome MLST typing methods for the routine use of WGS in outbreak situations

### Emerging MRSA strains

- Monitoring of the characteristics of novel and potentially emerging MRSA clones e.g. ST772-MRSA-V, and ST1-MRSA-IV which carry multiple resistance and virulence genes and have been involved in outbreaks in healthcare facilities

### CA-MRSA

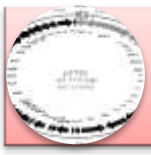
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

### LA-MRSA

- Investigation of MRSA from animal populations for the presence of *mecC* in order to determine if isolates harbouring this gene are a significant problem among LA-MRSA isolates Ireland or if the zoonotic spread of these MRSA strains are contributing to the burden of MRSA among humans

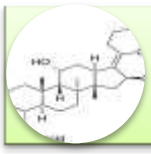
### MSSA

- Investigating the genotypes, virulence and antimicrobial resistance potential of MSSA isolates associated with BSI) and MRSA from BSIs in order to investigate why MSSA BSIs are increasing in Ireland while MRSA BSIs are decreasing



### Mupirocin resitance

- Investigation of the genotypes of Hi-MupR MRSA isolates and in-depth analysis of Hi-MupR-conferring plasmids



### Fusidic acid resistance

- Investigation of the genetic mechanism of fusidic acid resistance in MRSA in Ireland

### Linezolid resistance

- Investigation of linezolid resistance among MRSA, CoNS and VRE and particularly resistance encoded for by the *cfr* and *optrA* genes

# PUBLICATIONS

Below are abstracts resulting from these very successful collaborations which have been published or accepted for publication throughout the year.

**Significant Enrichment and Diversity of the Staphylococcal Arginine Catabolic Mobile Element ACME in *Staphylococcus epidermidis* Isolates From Subgingival Peri-implantitis Sites and Periodontal Pockets. O'Connor AM, McManus BA, Kinnevey PM, Brennan GI, Fleming TE, Cashin PJ, O'Sullivan M, Polyzois I, Coleman DC. Front Microbiol. 2018 Jul 12; 9: 1558.**

*Staphylococcus aureus* and *Staphylococcus epidermidis* are frequent commensals of the nares and skin and are considered transient oral residents. Reports on their prevalence in the oral cavity, periodontal pockets and subgingivally around infected oral implants are conflicting, largely due to methodological limitations. The prevalence of these species in the oral cavities, periodontal pockets and subgingival sites of orally healthy individuals with/without implants and in patients with periodontal disease or infected implants (peri-implantitis) was investigated using selective chromogenic agar and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Staphylococcus epidermidis* was predominant in all participant groups investigated. Its prevalence was significantly higher ($P$ = 0.0189) in periodontal pockets (30%) than subgingival sites of healthy individuals (7.8%), and in subgingival peri-implantitis sites (51.7%) versus subgingival sites around non-infected implants (16.1%) ($P$ = 0.0057). In contrast, *S. aureus*was recovered from subgingival sites of 0-12.9% of the participant groups, but not from periodontal pockets. The arginine catabolic mobile element (ACME), thought to enhance colonization and survival of *S. aureus*, was detected in 100/179 *S. epidermidis* and 0/83 *S. aureus* isolates screened using multiplex PCR and DNA microarray profiling. Five distinct ACME types, including the recently described types IV and V (I; 14, II; 60, III; 10, IV; 15, V; 1) were identified. ACME-positive *S. epidermidis* were significantly ($P$ = 0.0369) more prevalent in subgingival peri-implantitis sites (37.9%) than subgingival sites around non-infected implants (12.9%) and also in periodontal pockets (25%) compared to subgingival sites of healthy individuals (4.7%) ($P$ = 0.0167). To investigate the genetic diversity of ACME, 35 isolates, representative of patient groups, sample sites and ACME types underwent whole genome sequencing from which multilocus sequence types (STs) were identified. Sequencing data permitted ACME types II and IV to be subdivided into subtypes IIa-c and IVa-b, respectively, based on distinct flanking direct repeat sequences. Distinct ACME types were commonly associated with specific STs, rather than health/disease states or recovery sites, suggesting that ACME types/subtypes originated amongst specific *S. epidermidis* lineages. Ninety of the ACME-positive isolates encoded the ACME-*arc* operon, which likely contributes to oral *S. epidermidis* survival in the nutrient poor, semi-anaerobic, acidic and inflammatory conditions present in periodontal disease and peri-implantitis.

**Intra-Hospital, Inter-Hospital and Intercontinental Spread of ST78 MRSA From Two Neonatal Intensive Care Unit Outbreaks Established Using Whole-Genome Sequencing. Earls MR, Coleman DC, Brennan GI, Fleming T, Monecke S, Slickers P, Ehricht R, Shore AC. Front Microbiol. 2018 Jul 4;9:1485.**

From 2009 to 2011 [transmission period (TP) 1] and 2014 to 2017 (TP2), two outbreaks involving community-associated clonal complex (CC) 88-MRSA *spa* types t186 and t786, respectively, occurred in the Neonatal Intensive Care Unit (NICU) of an Irish hospital (H1). This study investigated the relatedness of these isolates, their relationship to other CC88 MRSA from Ireland and their likely geographic origin, using whole-genome sequencing (WGS). All 28 CC88-MRSA isolates identified at the Irish National MRSA Reference Laboratory between 2009 and 2017 were investigated including 20 H1 patient isolates, two H1 isolates recovered from a single healthcare worker (HCW) 2 years apart, three patient isolates from a second hospital (H2) and one patient isolate from each of three different hospitals (H3, H4, and H5). All isolates underwent DNA microarray profiling. Thirteen international isolates with similar microarray profiles to at least one Irish isolate were selected from an extensive global database. All isolates underwent Illumina MiSeq WGS. The majority of Irish isolates (25/28; all H1 isolates, two H2 isolates and the H3 isolate) were identified as ST78-MRSA-IVa and formed a large cluster, exhibiting 1-71 pairwise allelic differences, in a whole-genome MLST-based minimum spanning tree (MST) involving all Irish isolates. A H1/H2, H1/H3, and H1 HCW/patient isolate pair each exhibited one allelic difference. The TP2 isolates were characterised by a different *spa* type and the loss of *hsdS*. The three remaining Irish isolates (from H2, H4, and H5) were identified as ST88-MRSA-IVa and dispersed at the opposite end of the MST, exhibiting 81-211 pairwise allelic differences. Core-genome MLST and sequence-based plasmid analysis revealed the recent shared ancestry of Irish and Australian ST78-MRSA-IVa, and of Irish and French/Egyptian ST88-MRSA-IVa. This study revealed the homogeneity of isolates recovered during two NICU outbreaks (despite *spa* type and *hsdS* carriage variances), HCW involvement in the outbreak transmission chain and the strain's spread to two other Irish hospitals. The outbreak strain, CC88/ST78-MRSA-IVa, was likely imported from Australia, where it is prevalent. CC88/ST88-MRSA-IVa was also identified in Irish hospitals and was likely imported from Africa, where it is predominant, and/or a country with a large population of African descent.

**European external quality assessments for identification, molecular typing and characterization of *Staphylococcus aureus.* Deplano *et al., J Antimicrob Chemother* 73: 10; 2662–6**

***OBJECTIVES:***

We present the results of two European external quality assessments (EQAs) conducted in 2014 and 2016 under the auspices of the Study Group on Staphylococci and Staphylococcal Infections of ESCMID. The objective was to assess the performance of participating centres in characterizing Staphylococcus aureus using their standard in-house phenotypic and genotypic protocols.

***METHODS:***

A total of 11 well-characterized blindly coded S. aureus (n = 9), Staphylococcus argenteus (n = 1) and Staphylococcus capitis (n = 1) strains were distributed to participants for analysis. Species identification, MIC determination, antimicrobial susceptibility testing, antimicrobial resistance and toxin gene detection and molecular typing including spa typing, SCCmec typing and MLST were performed.

***RESULTS:***

Thirteen laboratories from 12 European countries participated in one EQA or both EQAs. Despite considerable diversity in the methods employed, good concordance (90%-100%) with expected results was obtained. Discrepancies were observed for: (i) identification of the S. argenteus strain; (ii) phenotypic detection of low-level resistance to oxacillin in the mecC-positive strain; (iii) phenotypic detection of the inducible MLSB strain; and (iv) WGS-based detection of some resistance and toxin genes.

***CONCLUSIONS:***

Overall, good concordance (90%-100%) with expected results was observed. In some instances, the accurate detection of resistance and toxin genes from WGS data proved problematic, highlighting the need for validated and internationally agreed-on bioinformatics pipelines before such techniques are implemented routinely by microbiology laboratories. We strongly recommend all national reference laboratories and laboratories acting as referral centres to participate in such EQA initiatives.

**Minimizing microbial contamination risk simultaneously from multiple hospital washbasins by automated cleaning and disinfection of U-bends with electrochemically activated solutions. Deasy EC, Moloney EM, Boyle M, Swan JS, Geoghegan DA, Brennan GI, Fleming TE, O'Donnell MJ and Coleman DC. J Hosp Infect. 2018 Nov;100(3):e98-e104.**

***BACKGROUND:***

Outbreaks of infection associated with microbial biofilm in hospital hand washbasin U-bends are being reported increasingly. In a previous study, the efficacy of a prototype automated U-bend decontamination method was demonstrated for a single non-hospital pattern washbasin. It used two electrochemically activated solutions (ECA) generated from brine: catholyte with detergent properties and anolyte with disinfectant properties.

***AIM:***

To develop and test a large-scale automated ECA treatment system to decontaminate 10 hospital pattern washbasin U-bends simultaneously in a busy hospital clinic.

***METHODS:***

A programmable system was developed whereby the washbasin drain outlets, U-bends and proximal wastewater pipework automatically underwent 10-min treatments with catholyte followed by anolyte, three times weekly, over five months. Six untreated washbasins served as controls. Quantitative bacterial counts from U-bends were determined on Columbia blood agar, Reasoner's 2A agar and Pseudomonas aeruginosa selective agar following treatment and 24 h later.

***FINDINGS:***

The average bacterial densities in colony-forming units/swab from treated U-bends showed a >3 log reduction compared with controls, and reductions were highly significant ($P<0.0001$) on all media. There was no significant increase in average bacterial counts from treated U-bends 24 h later on all media ($P>0.1$). *P. aeruginosa* was the most prevalent organism recovered throughout the study. Internal examination of untreated U-bends using electron microscopy showed dense biofilm extending to the washbasin drain outlet junction, whereas treated U-bends were free from biofilm.

***CONCLUSION:***

Simultaneous automated treatment of multiple hospital washbasin U-bends with ECA consistently minimizes microbial contamination and thus the associated risk of infection.

# POSTERS

The following posters were presented at National and International Conferences throughout the year.

Diverse SCC*mec-fusC* elements among distinct community-associated MRSA strains. Brennan *et al.,* ISSSI, Copenhagen

The role of *Staphylococcus aureus* colonisation of healthcare workers in nosocomial transmission of *S. aureus* to patients investigated using whole-genome sequencing. Earls *et al,* ISSSI*,* Copenhagen

Emergence and global spread of a multidrug-resistant, community-associated MRSA lineage from the Indian subcontinent. Steinig *et al.,* ISSSI, Copenhagen

An investigation of the prevalence and temporal dynamics of *Staphylococcus aureus* carriage among healthcare workers. Kearney *et al., Healthcare Infection Society meeting, Dublin*

An investigation of the role of *Staphylococcus aureus* colonisation of healthcare workers in nosocomial transmission of *S. aureus* to patients in an MRSA endemic setting using whole-genome sequencing: Summary of recruitment and results of phase 1 (May-October 2017). Kearney *et al.,* RCSI Research Day, Dublin

# RESOURCES

## Staff

During 2018 the staff working in the NMRSARL were

- Gráinne Brennan
- Tanya Fleming
- Paul Grier
- Fionnuala McGrath
- Ludmila Fadejeva
- Ciara Uí Mhuineachain

Dr. Anna Shore continued in her role as a Lecturer in Applied and Translational Microbiology and, in this role continued her involvement in the development of applied research in MRSA between the School of Dental Science, Trinity College and NMRSARL.

The role of Director was discharged in an honorary capacity by Dr. Brian O'Connell, Consultant Microbiologist, SJH. Professor Hilary Humphreys of the Royal College of Surgeons in Ireland and Beaumont Hospital continued in his role of Honorary Consultant to provide an external perspective to the activities and services provided by NMRSARL.

## Facilities

NMRSARL consists of three main laboratory areas, a Phenotyping Laboratory, a Genotyping Laboratory and a PCR Laboratory. The provision of a suitable computer system is a major requirement, both for monitoring isolates received and for detailed analytical work.

Along with the Central Pathology Laboratory in SJH, NMRSARL has been involved in procuring a new computer system for a number of years and as part of this procurement, the special requirements of NMRSARL have been noted. However, all systems investigated to date would require extensive modification to accommodate NMRSARL's needs.

## Finance

The budget allocated to the NMRSARL for the year to cover both pay and non-pay elements amounted to €361,131.

## Administration

The laboratory is located in St. James's Hospital and is administered within the Laboratory Medicine (LabMed) Directorate.

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